Claims

Sub A1

1. A method for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence, said method comprising:

(a) contacting a nucleic acid sequence, in the same or a separate reaction, with a first pair of PCR primers and a second pair of PCR primers under conditions that allow hybridization of said PCR primers to said nucleic acid sequence, said first pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, and said second pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, said PCR primers being characterized as follows:

(i) one of said first pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at a single non-complementary nucleotide in its 3'-terminal nucleotides 2-6; and

(ii) one of said second pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at one or more nucleotides in its 3'-terminal nucleotides 2-6;

(b) carrying out said amplification reactions; and

(c) detecting an amplification product as an indication of the presence, in said nucleic acid sequence, of said first allele of said polymorphic

sequence.

2. The method of claim 1, wherein the amplification reaction involving said first pair of PCR primers and the amplification reaction involving said second pair of PCR primers have different ranges of specificity.

3. The method of claim 2, wherein said ranges of specificity overlap.

4. The method of claim 3, wherein said amplification reaction involving said first pair of PCR primers and said amplification reaction involving said second pair of PCR primers together have a greater than 3000-fold range of specificity.

5. The method of claim 4, wherein said amplification reaction involving said first pair of PCR primers and said amplification reaction involving said second pair of PCR primers together have at least a 10,000-fold range of specificity.

Sub a2 6. The method of claim 1, wherein said one of said second pair of PCR primers includes at least two non-complementary nucleotides in its 3'-terminal nucleotides 2-6.

7. The method of claim 1, wherein said method is carried out to identify a single nucleotide polymorphism.

8. The method of claim 1, wherein each of said primers of said first and said second primer pairs that comprises a non-complementary nucleotide in

its 3'-terminal nucleotides 2-6 also comprises a universal primer binding site.

9. The method of claim 8, wherein said detecting step comprises amplification of said product of step (b) using a detectably labelled PCR primer that hybridizes to said universal primer binding site.

Sub A3

10. The method of claim 1, wherein each of said primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6 also comprises a unique hybridization tag.

11. The method of claim 10, wherein said detection step is facilitated by said hybridization tag.

Sub A4

12. The method of claim 11, wherein said detection step is carried out on a solid support to which a binding partner for each hybridization tag is immobilized.

13. The method of claim 12, wherein said solid support is a chip.

Sub A5

14. The method of claim 1, further comprising:

(a) contacting said nucleic acid sequence, in the same or a separate reaction, with a third pair of PCR primers and a fourth pair of PCR primers under conditions that allow hybridization of said PCR primers to said nucleic acid sequence, said third pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, and said fourth pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic

sequence, said PCR primers being characterized as follows:

(i) one of said third pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at a single nucleotide in its 3'-terminal nucleotides 2-6; and

(ii) one of said fourth pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at one or more nucleotides in its 3'-terminal nucleotides 2-6; and

(b) carrying out said amplification reactions; and

(c) detecting an amplification product as an indication of the presence, in said nucleic acid sequence, of said second allele of said polymorphic sequence.

15. A kit for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence, said kit comprising:

(a) a first pair of PCR primers and a second pair of PCR primers, said first pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, and said second pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, said PCR primers being characterized as follows:

(i) one of said first pair of PCR primers (a) being

complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at a single non-complementary nucleotide in its 3'-terminal nucleotides 2-6; and

(ii) one of said second pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequenc, (b) being non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at one or more nucleotides in its 3'-terminal nucleotides.

16. The kit of claim 15, further comprising:

(a) a third pair of PCR primers and a fourth pair of PCR primers, said third pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, and said fourth pair of PCR primers hybridizing to opposite strands of said nucleic acid sequence and bordering the position of said polymorphic sequence, said PCR primers being characterized as follows:

(i) one of said third pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at a single nucleotide in its 3'-terminal nucleotides 2-6; and

(ii) one of said fourth pair of PCR primers (a) being complementary at its 3'-terminal nucleotide to said second allele of said

polymorphic sequence, (b) being non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) being non-complementary to said nucleic acid sequence at one or more nucleotides in its 3'-terminal nucleotides 2-6.

17. The kit of claim 15, wherein said one of said primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6 also comprises a universal primer binding sequence.

18. The kit of claim 15, wherein said one of said primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6 also comprises a unique hybridization tag.

19. The kit of claim 18, wherein said kit further includes a solid support to which is immobilized a binding partner for each hybridization tag.

20. The kit of claim 19, wherein said solid support is a chip.